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Variations in oxidant-antioxidant status in chronic obstructive pulmonary disease

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a common disease affecting the lungs. COPD is one of the major cause of morbidity and mortality. Cigarette smoke, the pollutants and the exogenous oxidants increase the oxidative stress leading to depletion of antioxidant capacities in patients with COPD. Most of the inflammation during COPD is produced by the endogenous oxidants. The literature on pathogenesis of COPD reveals the hypothesis of imbalance between oxidants and antioxidants as important mechanism. The aim of the present study was to assess the role of an imbalance between oxidants and the antioxidant defence in COPD group (COPD smokers, COPD ex-smokers and COPD non-smokers). The present study shows the evaluation of oxidant markers like plasma *MDA* (Malondialdehde) and *NOx* (Nitric oxide) with antioxidant markers *SOD, GPx, CAT* and *Hcp* (Super oxide dismutase, Glutathione peroxidase, Catalase and ceruloplasmin) in COPD patients and control subjects. The oxidative markers was found to be significantly ($p<0.0001$) elevated in group of COPD patients when compared with the controls while the levels of antioxidant markers *SOD* and *CAT* levels get decreased. The *GPx* and serum *Hcp* levels get increased, showing a significant association ($p<0.0001$). Our results suggest that oxidative stress and antioxidative status plays animportant role in the pathogenesis of COPD.

KEYWORDS

COPD smokers;
Ex-smokers;
Non-smokers;
Oxidative stress;
Antioxidants.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by the gradual progression of irreversible airflow obstruction and increased inflammation in the airways of lung parenchyma. It is a systemic disorder that is associated with increase of inflammatory proteins in systemic circulation and it is a leading cause of morbidity and increasing mortality in developing countries[1-3]. The literature so far studied and experimental work have shown the evidence of imbalance between oxidants and antioxidants in presence of reactive oxygen species (ROS) leading to the development of COPD[4-6]. Oxidative stress in COPD is increased due to action of many exogenous oxidants present in the environment. The cigarette smoke and its components along with endogenous oxidants release inflammatory response in the lungs[7-10]. Antioxidative status in COPD is decreased as the oxidative burden gets inclined. Various studies have shown the antioxidativemarkers in

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plasma of COPD smokers are decreased with an increase in oxidative stress[11]. However there are controversial results regarding the studies conducted on oxidative and antioxidative stress imbalance in the pathogenesis of COPD.The sources of the increased oxidative stress in patients with COPD are derived from the increasedburden of oxidants present in cigarette smoke, increasing the amount of reactive oxygen species released from leukocytes, both in the airspaces and in the blood[12]. MDA levels and their end products have been extensively studied as a marker in pulmonary, systemic oxidative stress and in the pathogenesis of COPD. The levels of MDA gets altered due to the structural changes which occur on reaction with free radicals released from cigarette smoke and impair the function of cell membrane permeability by inactivating the receptor and enzymes[13].

NO is generated in COPD from the enzyme inducible NO synthase, which is expressed in macrophages and lung parenchyma of patients with COPD[14,15]. NO levels are depressed by cigarette smoking and oxidative stress, since NOx combines avidly with superoxide anions to form peroxynitrite leading to the increased levels of oxidative stress in COPD[16,17]. This kind of studies reflects that the evaluation of oxidative stress markerslike plasma MDA and NOx levels in biological samples can play a significant role in the increased burden oxidative stress in diseases like COPD and also as aclinical marker in the oxidative damage of chronic diseases[18-21].

The antioxidant systems includes enzymes like super oxide dismutase, catalase, glutathione peroxidase and proteins like albumin, bilirubin, ferritin, and ceruloplasmin along with molecules like uric acid, reduced glutathione and ubiquinol. Deficiency of antioxidant capacity leads to high oxidative stress which is an increasingevidence in relation to smoking.The studies conducted on COPD by Rahman et.al[22] have shown decrease levels of antioxidant enzymes. The other reason for reduction of antioxidant levels in smokers is also due to depletion of plasma protein Sulphadryls[23-25].

The present study is aimed to evaluate the imbalance between oxidant and antioxidant status in COPD patients (COPD smokers, COPD ex-smokers and COPD non-smokers) in comparision with the healthy controls as the existing hypothesis involved in the pathogenesis of COPD

MATERIALS AND METHODS

Study population

The Institutional ethical clearance was obtained to carry out the study.Special case proformas and consent forms for COPD and healthy controls subjects have been prepared to collect the detailed case histories and written consent from the cases willing to be recruited for the study had been taken

A total of two hundred and fifty (mean age n=250) COPD cases were taken from Government Chest Hospital, Irranuma, Hyderabad which is one of the reputed hospitals in Andhra Pradesh, where patients from different socioeconomic strata are referred.The cases which were diagnosed by spirometry, chest X-ray and confirmed by pulmonologists were considered for the study.The Spirometric classification of severity of COPD including four stages: stage I, mild; stage II, moderate; stage III, severe; stage IV, very severeCOPD (cases with a history of cigarette smoking, cough, sputum, persistence dyspnea, acute excaberations with their profession and other COPD risk factors were included for the study). Emphasis is given for the details of the epidemiological variables like age, sex, BMI, addictions such as smoking, Pack years in ex-smokers and other clinical profiles with physiological characteristics are shown in TABLE 1.One hundred ninety seven of the COPD patients had a history of smoking, forty five of them had a past history of smoking and had stopped smoking at least two year before their involvement in the study and nine COPD patients were non-smokers. The information obtained in the proforma from COPD non-smokers showed that by profession two of them were labours working in industry, farmer, two welders, washerman and painters who were suffering with COPD from 2-4 years.An equal number of Clinically healthy controls and free of overt disease with same geographic background and similar socioeconomic status (n=250, mean age 41.79 ± 15.76 years; 9 female, 239 males, BMI 28.352 ± 8.014 kg/m2) were considered for the present study.

Oxidative stress was assessed in plasma through the determination of the levels of biomarkers such as MDA and nitric oxide in COPD group and in control subjects. Antioxidant status was evaluated by the quantitative analysis of super oxide dismutase, (SOD), Glutathione peroxidase (Gpx), Catalase (CAT) and Ceruloplasmin (Hcp), using spectrometric methods.

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TABLE 1 : Clinical and physiological characteristic of COPD group

Clinical and physiological parameters	Values
Age, years	59,32 ± 10,29
Male/female,n	239/11
Body mass index, kg/m	219,660 ± 4,990
Smoking status: smokers/ex-smokers/nonsmokers, n	197/45/09
Pack-years in smokers/ exsmokers	88,2±19.768/75,8 ± 15,262
GOLD stage:I/II/III/IV, n	73/48/121/08
FVC,% predicted	78,122±10,196
FEV1, % predicted	42,102±10,962
FEV1/FVC, %	54,163± 6.502

Methods

Determination of oxidative markers plasma nitric oxide & MDA

The nitrite and nitrate of nitric oxide a biomarker of oxidative stress has been assessed in plasma. The levels were measured spectrophotometrically by the method of Green etal[26]. The absorbance were read at 542 nm by using Griess reagent forming a coloured complex. The concentration of plasma nitric oxide was calculated using the calibration curve. The concentration of NOx was expressed in µm/ML of plasma.

The other marker MDA were measured spectrometrically in terms of thiobarbituric acid (TBA). The concentration of TBA were measured by using the modified method ofGavino et.al[27]. The absorbance were read at 532 nm corresponding to the colored complex formed between the MDA and thiobarbituric acid (TBA). The concentration of TBARS was calculated using the MDA concentration and using a calibration curve. The concentration of MDA was expressed in nmol/mL of plasma.

Determination of antioxidant markers, erythrocyte (RBC Lysate) super oxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and serum ceruloplasmin (Hcp)

We evaluated the antioxidant enzyme super oxide dismutase in the RBC lysate using a spectrophotometric method of McCord and Fridovich(1969) using xanthine[28]. SOD catalyzes the dismutation of the superoxide radical (O_2-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2) into the inter-membrane space or mitochondrial matrix, and thus provides an important defense against the toxicity of superoxide radicals.The method of Xanthine-Xanthine (XOD) are used to generate superoxide radical. 2-(4-iodophenyl)-3-(4-nitrophenol)-5phenyltetrazolium chloride (INT) reacts with superoxide radical to form a red formazan dye (detector). SOD inhibits the formation of formazan dye. Absorbance was monitored at 505nm. The concentration of formazan was calculated using the SOD concentration and using a standard curve previously prepared. The levels of SOD were expressed in U/Hb.

Glutathione peroxidase (GPx) levels were moni-

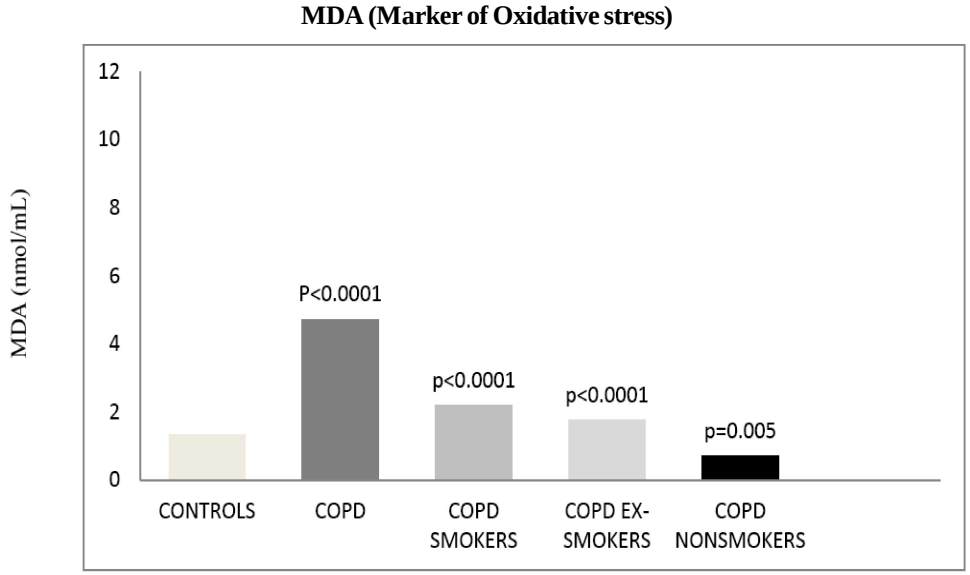

Figure 1 : Levels of malondialdehyde (MDA) in plasma from control subjects and COPD patients. Values are expressed as mean ± SD. p<0.0001 significantly different between controls and COPD group. p<0.0001 significantly different between controls and COPD smokers. p<0.0001 significantly different between controls and Ex-smokers, p=0.005 significantly different in controls and COPD non-smokers

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tored by the method of Beutler et.al. (1975) In brief, the Glutathione peroxidase (GPx) catalyses the conversion of reduced glutathione (GSH) to oxidizes glutathione (GSSG) and hydrogen peroxide in the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted back to reduced glutathione with oxidation of NADPH to NADP+[29]. The decrease in absorbance at 340nm, indicative of the extent of oxidation of NADPH to NADP+, was estimated as activity of GSH-Px. Results were defined as 1U/gHb.

The activity of catalase, a cellular antioxidant enzyme, present in biological samples (RBC lysate) is estimated by measuring its ability to decompose hydrogen peroxide, formed due to metabolic reactions, into water and oxygen described by Aebietal (1984)[30]. Hydrogen peroxide has absorbance at 240nm and the decrease in the O.D. is an indicator of the decomposition of hydrogen peroxide by catalase. The activity in the sample was expressed as U/gm Hb.

The protein ceruloplasmin was measured in serum by using the method of Ravinetal[31]. It can catalyze the oxidation of some polyamines and its action on paraphenylenediamine (PPD) was used as a measure of amount present in serum. The absorbance was measured at 540nm and the units were expressed as mg/dl.

Stastical analyses

The results are expressed as mean ± SD of the concentrations of plasma and serum parameters were evaluated. The clinical and physiological parameters was chosen for normal distribution. Comparisons were tested among the groups using unpaired t-test or Mann-Whitney U-test or unpaired t-test were tested for comparisions between two groups. $p<0.05$ was considered stastically significant

RESULTS

Oxidative stress and antioxidant status

The plasma levels of oxidants MDA, NOx and antioxidants SOD, GPx, CAT and serum Hcp levels of COPD patients (COPD, COPD smokers, COPD ex-smokers and COPD non-smokers) and control subjects are shown in TABLE 2. The markers of oxidative stress MDA was found to be significantly higher ($p<0.0001$) in COPD group when compared with control subjects Figure 1. The levels of TBARS found to be significantly different between COPD smokers, COPD ex-smokers($p<0.0001$) and COPD non- smokers ($p=0.005$) and Controls.

The plasma nitric oxide levels (NOx) between controls and COPD, COPD smokers and COPD non smokers were found to significant ($p<0.0001$) but we found no significant differences among the controls and COPD Ex-smokers Figure 2.

The anti-oxidants superoxide dismutase (SOD), in the erythrocyte lysate showed a significant decrease in COPD group in comparison with the controls ($p<0.0012$) Further there is an significant decrease in COPD smokers, COPD ex-smokers and COPD non-smokers ($p<0.0001$) Figure (a). The glutathione peroxidase (GPx) levels were found to be significantly in-

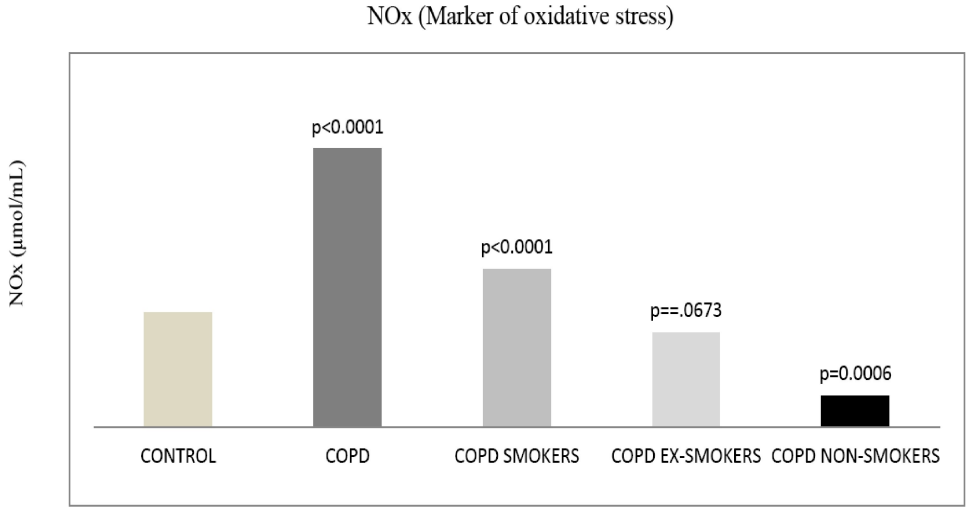

Figure 2 : Levels of nitric oxide (NOx) in plasma from control subjects and COPD patients. Values are expressed as mean ± SD. p<0.0001 significantly different between controls and COPD. p<0.0001 significantly different between controls and COPD smokers.p<0.0001 significantly different between controls and COPD non-smokers

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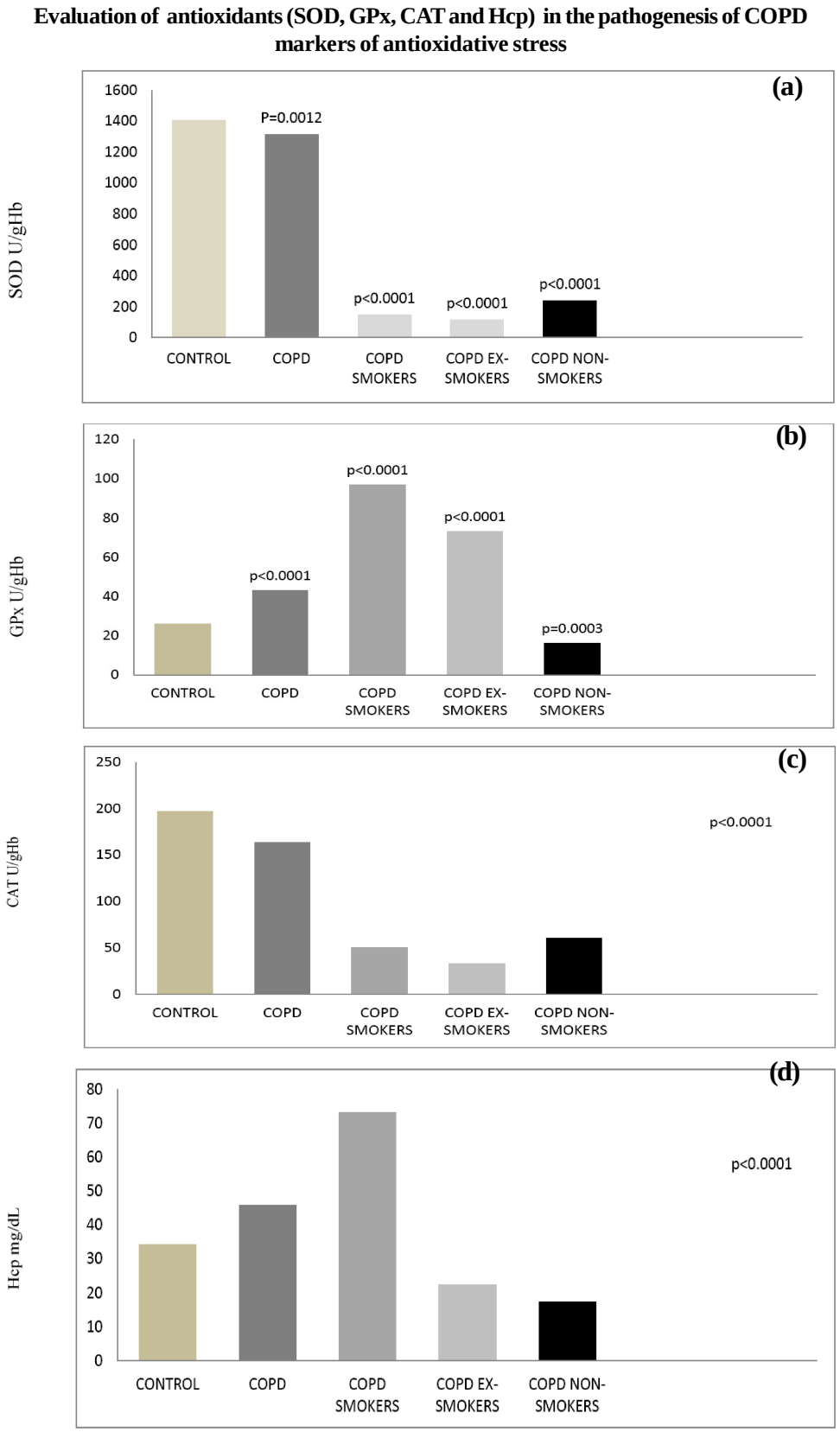

Figure 3 : Levels of (a). super oxide dismutase (SOD), (b). glutathione peroxidase (GPx), (c). catalase (CAT) and serum (d). ceruloplasmin (Hcp) in control subjects and COPD patients. Values are expressed as mean ± SD. COPD patients had a significant (p<0.0001) decrease in antioxidants (SOD and CAT) and increase in (GPx and Hcp) levels (p<0.0001) in comparision with the control group

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TABLE 2 : Plasma MDA, NOx levels and antioxidant status of the study groups

	Controls (n=250)	COPD (n=250)	COPD smokers (n=197)	COPD ex-smokers (n=45)	COPD non-smokers n (n=09)
Oxidants					
MDA (nmol/mL)	1.342±0.652	4.734±3.572	2.216±0.996	1.786±0.682	0.734±0.268
NOx(µmol/ml)	1.123±0.699	2.715±1.552	1.546±0.142	0.929±0.263	0.311±0.026
Anti-oxidants					
SOD(U/gHb)	1406.94±126.75	1312.53±439.23	149.53±24.69	116.42±17.63	239.12±.76.2
GPx(U/gHb)	26.134±8.197	43.145±11.26	96. 99±19.183	73.12±16.612	16.134±2.19
CAT(U/gHb)	197.18±67.47	163.94±60.37	50.34±22.19	32.94±18.06	60.26±27.28
Hcp(mg/dl)	34.327±9.132	45.97±11.194	73.27±24.01	22.45±11.45	17.26±5.24

All the data are expressed as mean ± SD

creased between COPD smokers, COPD ex-smokers ($p<0.0001$) and COPD ex-smokers ($p=0.0003$) when compared with controls Figure (b.)The catalase (CAT) levelsbetween controls and COPD group are significantly decreased ($p<0.0001$) Figure (c).

Serum ceruloplasmin (Hcp) levels had a significant ($p<0.0001$) increase in antioxidant status of COPD patients when compared with the control subjects. Significant difference were obtained between COPD smokers, COPD ex-smokers and COPD non-smokers($p<0.0001$) Figure (d).

DISCUSSION AND CONCLUSIONS

The exogenous and endogenous sources of ROS mechanism gets imbalanced in inflammatory diseases like COPD during which oxidative stress markers gets increased with decrease in antioxidant levels. As the ROS levelsgets disturbed there is imbalance in oxidants and antioxidant mechanisms resulting in altered pathological conditions. The aim of the present study was to evaluate and to compare the oxidative stress markers in plasma MDA and NOx levels and the antioxidants SOD, GPx, CAT and Hcp in COPD group (COPD smokers, COPD Ex-smokers and COPD non-smokers) with healthy controls. Our results suggest that oxidative stress and antioxidants status play a vital role in pathophysiological changes involved in COPD.

The primary consequence of oxidative stress occurs on the cell membrane of lipids leading to cell damage or complete cell death. The evaluation of plasma products like lipid perioxidation (MDA) and nitric oxide are helpful asmarkers to examine the oxidative stress in disease condition.In our studies we found a significant association of plasma MDA levels between controls and COPD groups, Cristovaoetal[32] observed that MDA levels were increased in COPD, COPD non smokers and COPD ex-smokers in comparison with the controls as our studies show the similar results. However there are other studies which show no correlation of TBARS in COPD associated with cigarette smoking (Atluntas 2003)[33].

Nitric oxide is used as marker for oxidative stress and in other inflammatory diseases. Petruzzelli S etal[34] have shown that Smoking increases directly Peroxy nitrite formed by the production of NO with superoxide anion. It hasbeen observed in this studythat nitric oxide levels gets increased in COPD group but we did not find significant difference in the levels of plasma nitric oxide among COPD controls and COPD ex-smokers. Some studies have shown decline in nitric oxide levels when compared with the healthy controls[35-37]. Over all we found that nitric oxide levels increases in smoking associated with COPD as proved in earlier studies carried out byauthors CliniE.etal[38]. The increase in lipid peroxidation products and plasma nitric oxide levels in COPD patients, assist the hypothesis of oxidative stress associated with the disease.

In addition, we observed the significant differences in the antioxidant status between controls and COPD group. The antioxidant status gets decreased as the levels of oxidative stress marker increases. SOD, GPx, CAT and Hcp are regarded as common antioxidants present in the blood. We found the levels of SOD get decreased in COPD group when compared with control subjects.The levels of SOD were checked in smokers with COPD, COPD ex-smokers and COPD non-smokers when compared with the controls and showed

low levels of SOD with a significant association($p<0.0001$). Previous studies states the same results which are in agreement with our obervations. Kim SH etal (2009)[39] RaghunathRaietal (2006)[40] and M.K Dagaetal (2003)[41] observed the similar decrease levels in smokers and COPD subjects when compared with the controls. However higher levels of SOD were observed by the authorR.P.Bowler et.al 2006[42] where theextracellular SODgets significantly elevated in the lungs of COPD patients.

In our studies, levels of Catalase (CAT) were found to be decreasedwith a significant association ($p<0.0001$) in comparison with the COPD group and controls. Tavilani H.etal (2012)[43] studies have observed the levels of catalase were lower in COPD patients and smokers when compared with non-smokers in accordance with our studies. Few studies on the activity of catalase had shown no alterations in the levels of catalase in COPD patients when compared with controls[44].

Serum Ceruloplasmin (Hcp) levels were found to be elevated in our studies with a significant association ($p<0.0001$) in comparison with the COPD and controls. These results were in accordance with the recent study carried out in India with serum ceruloplasmin and ferrioxidase activity in COPD patients by VivekAmbade.etal[45]. However TavilianiH.etal did not find any significant levels in plasma Hcp levels in COPD patients, smokers and non-smokers[43].

We further noted that the COPD patients were not on any oxygen therapy. The age limit of the patients were above 45 years. The oxidative stress levels MDA, NOx and antioxidant status were compared in relation with the age among COPD subjects and controls and there was no significant differences observed. These results obtained were in observance with the other studies in relation to age[46]. Hence in our study there is a significant association of oxidative stress markersMDA and NOx levels along with antioxidants GPx, Hcp, showing increased levels where as SOD and Catalase levels were found to be low in COPD group in comparison with healthy controls which is similar to astudy conducted recently in relation with oxidant and antioxidants status in COPD[47]. Further studies are needed to confirm the role of oxidants and antioxidant status in COPD patients.

To conclude, this study showed the elevated levels of MDA and NOx and decrease in antioxidants SOD and GPx with CAT and Hcp showing increased levels in plasma and serum of COPD patients. This significance was observed among COPD smokers, COPD exsmokers and COPD ex-smokers butplasma NOx showing no significance when compared with the controls. This imbalance between the oxidants and antioxidants in COPD patients can serve as a biochemicalmarkers in the diagnosis of the disease and help in intervention of theraupetic drugs for treatment of COPD.

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